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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Richard S. Blumberg et al.
Serial No: 09/122,144
Filed: July 24, 1998
For: RECEPTOR SPECIFIC TRANSEPITHELIAL TRANSPORT OF THERAPEUTICS

Examiner: G. Ewoldt
Art Unit: 1644

Certificate of Mailing Under 37 C.F.R. §1.8

I hereby certify that this correspondence is being deposited with the United States Postal Service as First Class Mail in an envelope addressed to the Commissioner for Patents, Washington, DC 20231, on the 5th day of June, 2001.

Alan W. Steele

Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

DECLARATION MADE UNDER 37 C.F.R. §1.132

1. This declaration in support of the above-referenced application is made by Alan J. Bitonti, Ph.D., Vice President for Research for Syntonix Pharmaceuticals, the assignee for the application.

2. I received my Ph.D. in pharmacology from the Ohio State University in 1978. From 1978 to 1981 I was a postdoctoral fellow in biochemistry and enzymology at the National Institutes of Health, Bethesda, MD. After completing my fellowship I joined Merrell-Dow Pharmaceuticals, which later became Hoechst Marion Roussel, where I remained until 1999. In 1999 I joined Syntonix Pharmaceuticals as their first hired scientist. I am currently Vice President for Research for Syntonix Pharmaceuticals.

3. My research expertise is in animal studies in areas including pharmacology, biochemistry, and cancer. I designed and conducted the animal experiments with EpoFc which are described below.

4. The Capon patent (U.S. 5,428,130) teaches Fc conjugates have prolonged half-lives but teaches nothing about transport across epithelial barriers. There is no motivation to increase the half-life of agents delivered to the periphery of the lung, where most uptake is believed to occur due to the immense surface area and the infinitesimal thickness of the air/blood barrier presented by the pulmonary epithelium and vascular endothelium, because clearance from the periphery is nearly absent. Thus, most pulmonary delivery, especially of larger proteins which generally have slower transport across an epithelium, is dependent on the efficient delivery of the administered agent to the deep lung in order to take advantage of the uniquely prolonged residence time in that portion of the lung.

5. A number of published studies have reported an inverse relationship between molecular size (molecular weight, MW) and absorption of agents administered via the pulmonary route. Absorption can be expressed in various terms, including permeability coefficient, bioavailability, lung clearance, and 1/time to maximal concentration in the plasma. All else being equal, smaller molecules are generally absorbed more readily from the pulmonary tract than larger molecules. Matsukawa Y et al., Size-dependent transport across rat alveolar epithelial cell monolayers. *J Pharm Sci* 86(3):305-9 (1997); Kobayashi S et al., Permeability of peptides and proteins in human cultured alveolar A549 cell monolayer. *Pharm Res* 12(8):1115-9 (1995); Effros RM et al., Measurements of pulmonary epithelial permeability in vivo. *Am Rev Resp Dis* 127:S59-65 (1983); Komada F et al., Intratracheal delivery of peptides and protein agents: absorption from solution and dry powder by rat lung. *J Pharm Sci* 83(6):863-7 (1994); Qiu Y et al., Absorption and bioavailability of inhaled peptides and proteins. In: Inhalation and Delivery of Therapeutic Peptides and Proteins, AL Adjei and PK Gupta, eds., Lung Biology in Health and Disease, Vol. 107, Marcel Dekker, NY, 1997, pp.89-131; Yu J et al., Pulmonary drug delivery: physiologic and mechanistic aspects. *Crit Rev Therap Drug Carrier Sys* 14(4):395-453 (1997); Byron PR et

al., Drug delivery via the respiratory tract. *J Aerosol Med* 7(1):49-75 (1994). Thus smaller peptides and proteins such as luteinizing hormone-releasing hormone (LHRH, MW 1 kDa), parathyroid hormone (PTH, MW 4.1 kDa), insulin (MW 5.7 kDa), and even human growth hormone (HGH, MW 22 kDa) are efficiently absorbed by pulmonary delivery relative to larger proteins such as human chorionic gonadotropin (HCG, MW 30 kDa), alpha-1-antitrypsin (MW 45 kDa), and albumin (MW 68 kDa). In this respect it would not be expected that conjugation of two molecules of erythropoietin (Epo, each with MW 30 kDa) to a macromolecule the size of Fc (MW ca. 50 kDa for homodimeric hinge, CH2 and CH3) would be advantageous to delivery across the pulmonary epithelium.

It would arguably be expected to be more advantageous to reduce the size (MW) of the agent or antigen delivered to the lung because the art teaches that transport across the epithelium is inversely related to MW. Thus, for example, for pulmonary administration it would appear to be intuitively advantageous to use a minimally sized biologically active fragment of Epo rather than a much larger Epo/Fc conjugate.

6. A number of published studies investigating the absorption of agents administered via the pulmonary route have reported the importance of delivery to the periphery, as opposed to central portions, of the lung when systemic absorption is desired. Newman SP et al., Understanding regional lung deposition data in gamma scintigraphy. *Resp Drug Del* VI:9-15 (1998). Generally, deep lung delivery is desirable for optimal absorption via the respiratory epithelium. High values of peripheral lung zone/central lung zone deposition ratio have been reported to be required to optimize the absorption of inhaled peptides. *Ibid.* Factors which enhance delivery to the peripheral lung include maximum degree of inspiration, slow flow inhalation, more distal point of introduction into the respiratory tract, inhaled particle diameter about 0.5 to 5 micrometers, and healthy lungs.

Even with all these factors optimized, the art teaches that pulmonary administration of aerosolized agents typically achieves no more than about 4-8 percent overall delivery efficiency

for a protein the size of insulin. Patton JS et al., Pulmonary absorption and metabolism of peptides and proteins. *Resp Drug Del* VI:17-24 (1998).

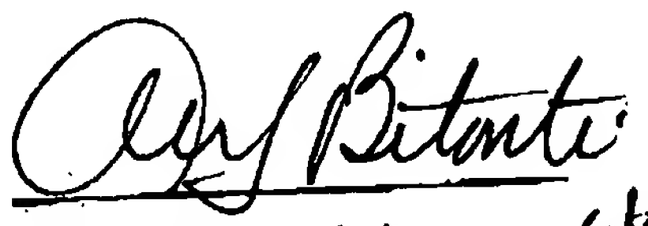
7. In striking contrast to these published observations, then, are the results of a study I designed and performed in which cynomolgus monkeys were treated with EpoFc. In this set of experiments monkeys were intubated and ventilated under positive pressure while in an "iron lung" in order to establish lung volumes (vital capacity, VC) and control depth of inspiration (% VC). Gamma scintigraphy was performed using administered ^{99}Tc-DTPA labeled aerosols containing EpoFc to measure the peripheral and central delivery under conditions of 75% VC versus 20% VC (See Figure 1). A 20% VC corresponds to normal depth ("tidal") inspiration, while a 75% VC corresponds to a near-maximal but straumatic forced inspiration. Figure 1 shows that 75% VC administration effectively delivered aerosol to the peripheral lung zones, while 20% VC administration delivered aerosol predominantly to central lung zones.

All animals receiving EpoFc by this method had measurable levels of EpoFc in the serum. Surprisingly, however, the serum concentration of EpoFc in animals receiving nebulized EpoFc at 20% VC greatly and consistently exceeded corresponding serum concentrations for animals receiving nebulized EpoFc at 75% VC (See Figure 2). Thus it was found that EpoFc delivered by aerosol predominantly to central lung zones yielded serum levels of EpoFc far in excess of what would be expected in view of the studies and reviews cited above. Significantly, bioavailability of EpoFc following aerosolized EpoFc at 20% VC was reproducibly in the range of 10-15 percent.

8. In summary, it should be apparent that the state of the art in pulmonary delivery of proteins for systemic uptake suggests that (1) smaller molecules are desirable over larger ones because of the inverse relationship between MW and bioavailability; (2) peripheral lung zone delivery is required for efficient transfer; and (3) delivery efficiency is low, on the order of 5%. In contrast, we have demonstrated in an in vivo study in primates that aerosolized EpoFc, with a MW much greater than insulin, has an unusually high bioavailability, even when administered to

the central zones of the lung. There would be no reason to expect this result from the teaching of the Capon patent, alone or in combination with any other reference.

Respectfully submitted,



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